ACTA SCIENTIFIC DENTAL SCIENCES (ISSN: 2581-4893)

Volume 3 Issue 5 May 2019

Research Article

Evaluation of Expression of Glut-1 in Metastatic and Non-Metastatic Oral Squamous Cell Carcinoma

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Received: March 05, 2019; **Published:** April 18, 2019

Abstract

Introduction and Objective: During carcinogenesis, uncontrolled cell proliferation leads to movement of cells away from blood vessels. Oxygen and glucose transport to the inner tumor cells is through diffusion across basement membrane and through peripheral tumor cell layers. Glut-1 glucose transporter is a transmembrane glycoprotein involved in Na+ independent transport of glucose into cells. Cellular homeostasis is maintained through an adaptive up regulation of Glut-1, as a result of hypoxia. Thus, the study aims at evaluating the relationship between OSCC and hypoxia biomarker such as Glut-1 and their prognostic significance.

Methods: The study was undertaken as a retrospective comparative study. The study comprised of two groups of 30 each, one group comprising of cases with histological evidence of lymph nodes metastasis and the other group of cases without histological evidence of lymph node metastasis. Paraffin embedded blocks of above mentioned 60 cases were retrieved. Glut-1 Immunohistochemical staining was performed in each case on the sections of primary tumor. Also, Hematoxylin and Eosin staining of primary tumor sections was done following which the sections were evaluated.

Results: Pearson's chi square test showed that there was statistically significant correlation (p = 0.001) between grade of Glut-1 expression and metastasis as around 96.7% cases were showing grade 3 of Glut-1 expression in the metastatic cases and 60% cases were showing grade 3 of Glut-1 expression in non-metastatic group.

Conclusion: The results of the present study indicate that immunoexpression of Glut-1 is stronger in metastatic cases of OSCC than cases without metastasis.

Keywords: Glut-1; Immunohistochemistry; Metastasis; Oral Squamous Cell Carcinoma; Prognosis; Staging

Introduction

During initial stages of carcinogenesis, uncontrolled cell proliferation, moves tumor cells away from blood vessels and there is reduced oxygen and nutrient supply. Oxygen and glucose transport to the inner tumor cells is through diffusion across basement membrane and through peripheral tumor cell layers. Partial oxygen pressure becomes very low away from blood vessels. This leads to hypoxia and glucose shortage in the inner mass of growing tumor [1].

The role of genes expressed in response to anaerobic conditions has been shown in studies related to the adaptive response of mammalian cells to hypoxia. Glut-1 glucose transporter is a transmembrane glycoprotein involved in Na+ independent transport of glucose into cells. Cellular homeostasis is maintained through an adaptive upregulation of Glut-1, whose expression is increased as a result of hypoxia. Glut-1 positivity in malignant

cells revealed by immunohistochemistry indicates increased proliferative activity, energy requirements and aggressive behavior [2].

Thus, according to earlier studies of Glut-1 expression in OSCC, Glut-1 maybe considered to be a negative biomarker for prognosis in OSCC [3-5]. Glut-1 immunoexpression has been studied in oral epithelial dysplasia, oral squamous cell carcinoma and verrucous carcinoma [5]. In previous studies, Glut-1 expression has been compared with respect to clinical staging and histological grading of squamous cell carcinoma. However, there are very few studies which attempt to correlate Glut-1 expression with lymph node metastasis in oral squamous cell carcinoma. Thus, the study aims at evaluating the relationship between OSCC and hypoxia biomarker such as Glut-1 and their prognostic significance.

Materials and Methods

Study design

The study was undertaken as a retrospective comparative study. The study comprised of two groups of thirty each, one group comprising of cases with histological evidence of lymph nodes metastasis and the other group of cases without histological evidence of lymph node metastasis. Paraffin embedded blocks of above mentioned sixty cases were retrieved from archives of Department of Oral and Maxillofacial Pathology and Microbiology at Manubhai Patel Dental College, and Department of Histopathology, Kailash Cancer Hospital, Munisevashram, Goraj. The data regarding tumor staging was also retrieved. The sections of primary tumors of each case were examined histologically after hematoxylin and eosin staining. Microscopic grade of the tumor was determined using WHO grading system. Table 1 and 2 show the demographic data of cases included in group 1 (Table 1) and in group 2 (Table 2).

No	Age	Gender	Site	Histopathologic grading	TNM staging
1	38	Male	Alveolus	Moderately differentiated	IV
2	75	Male	Left buccal mucosa	Moderately differentiated	IV
3	40	Male	Left lateral tongue	Moderately differentiated	III
4	55	female	Right lateral tongue	Moderately differentiated	IV
5	63	Male	Middle 1/3rd of mandible	Moderately differentiated	IV
6	50	Male	Left buccal mucosa	Poorly differentiated	III
7	45	Male	Left buccal mucosa	Moderately differentiated	III
8	55	Female	right lateral tongue	Moderately differentiated	IV
9	60	Male	Left lower lip	Moderately differentiated	IV
10	52	Male	Left buccal mucosa	Moderately differentiated	III
11	59	female	Left buccal mucosa	Moderately differentiated	IV
12	73	Male	Left buccal mucosa	Moderately differentiated	IV
13	38	Male	Left buccal mucosa	Moderately differentiated	IV
14	63	Male	Left lateral tongue	Moderately differentiated	III
15	55	Male	Right buccal tongue	Moderately differentiated	III
16	36	male	Right buccal mucosa	Moderately differentiated	IV
17	35	Male	Right buccal mucosa	Moderately differentiated	IV
18	53	female	Left lateral tongue	Moderately differentiated	III
19	77	Male	Left buccal mucosa	Poorly differentiated	IV
20	50	Male	Right alveolus	Moderately differentiated	III
21	40	Male	Right buccal mucosa	Moderately differentiated	IV
22	60	Male	Left lateral tongue	Moderately differentiated	IV
23	51	Male	Right lateral tongue	Moderately differentiated	IV
24	38	Male	Right lateral tongue	Moderately differentiated	IV
25	55	Male	Left buccal mucosa	Moderately differentiated	IV
26	42	Male	Left buccal mucosa	Moderately differentiated	IV
27	28	Male	Left lateral tongue	Moderately differentiated	III
28	37	Male	Left buccal mucosa	Moderately differentiated	IV
29	48	Male	Right tongue and floor of the mouth	Poorly differentiated	IV
30	45	Male	Left buccal mucosa	Moderately differentiated	III

Table 1: Clinical details of the patients in group 1 (Cases showing locoregional metastasis).

No	Age	Sex	Site	Histopathologic grading	TNM staging
1	48	Male	Right buccal mucosa	Moderately differentiated	IV
2	50	Male	Left buccal mucosa	Moderately differentiated	IV
3	52	Male	Left buccal mucosa	Well differentiated	I
4	32	Male	Right buccal mucosa	Moderately differentiated	IV
5	60	Female	Right lower alveolus	Moderately differentiated	IV
6	57	Male	Right buccal mucosa	Moderately differentiated	IV
7	48	Male	Right lateral tongue	Moderately differentiated	I
8	63	Male	Right lower alveolus	Moderately differentiated	II
9	50	Male	Left upper lip	Moderately differentiated	II
10	40	Male	Right lateral tongue	Poorly differentiated	II
11	48	female	Right lateral tongue	Moderately differentiated	II
12	37	Male	Left buccal mucosa	Moderately differentiated	I
13	60	Male	Left buccal mucosa	Moderately differentiated	I
14	55	female	Left buccal mucosa	Moderately differentiated	IV
15	47	Male	Left buccal mucosa	Moderately differentiated	II
16	40	Male	Left buccal mucosa	Moderately differentiated	II
17	38	Male	Left lateral tongue	Moderately differentiated	III
18	43	Male	Right buccal mucosa	Moderately differentiated	II
19	44	Male	Right lateral tongue	Moderately differentiated	III
20	46	Male	Right lateral tongue	Moderately differentiated	II
21	38	Male	Right buccal mucosa	Poorly differentiated	II
22	50	Male	Right buccal mucosa	Moderately differentiated	II
23	51	Male	Right alveolus	Moderately differentiated	II
24	45	Male	Left buccal mucosa	Well differentiated	I
25	42	Male	Right lateral tongue	Moderately differentiated	II
26	40	Male	Right lateral tongue	Well differentiated	III
27	65	Male	Right lateral tongue	Moderately differentiated	II
28	52	female	Right upper alveolus	Moderately differentiated	IV
29	57	Male	Right lateral tongue	Moderately differentiated	I
30	58	Male	Right buccal mucosa	Moderately differentiated	II

Table 2: Clinical details of the patients in group 2 (Cases without locoregional metastasis).

Immunohistochemistry

Formalin-fixed, paraffin-embedded tissue sections from each case were deparaffinized in xylene followed by rehydration through decreasing grades of alcohol. Sections were rinsed with phosphate buffered saline (PBS). The slides were arranged in a staining container. 300 ml of 10 mM citrate buffer, pH 6.0 was poured into the staining container and incubated at 95 - 100°C for 10 minutes in the declocking chamber for antigen retrieval. The staining container was then removed and brought to room temperature allowing the slides to cool for 20 minutes.

The sections were rinsed with PBS. Endogenous peroxidase was blocked by incubating sections in 3% H_2O_2 solution in methanol at room temperature for 10 minutes. The sections were again rinsed with PBS. 100 µl of appropriately diluted (in antibody dilution buffer, e.g. 0.5% bovine serum albumin in PBS) anti Glut-1 antibody (Mouse monoclonal antibody, Clone: SPM498, Biogenex, Fremont,

CA, USA) was poured on the sections. Following this the sections were incubated in a humidified chamber at room temperature for 1h. After one hour the sections were rinsed with PBS. 100 µl of secondary antibody to the sections on the slides and incubation in an autostainer at room temperature for 30 minutes was done. The sections were rinsed with PBS. 100 µl of horse reddish peroxidase (HRP) conjugate was poured onto the sections and incubated in an autostainer at room temperature for 30 minutes. The sections were rinsed with PBS. Freshly made substrate, 3,3' Diaminobenzidine (DAB) solution was added to the sections. Color development was allowed for < 5 minutes until the desired color intensity was reached. The sections were thoroughly washed with PBS. Counterstaining was done by immersing the sections in Mayer's Hematoxylin for 1 - 2 minutes. The sections were rinsed under running tap water for two minutes. Dehydration of the tissue sections was done by immersing through increasing grades of alcohol. The sections were mounted using DPX mounting medium, following clearing in xylene.

Assessment of Glut-1 immunoexpression

Observation of the intensity of Glut-1 immunoexpression in the tissue sections was performed under 400x magnification of light microscope (Magnus MLX-B Plus, Olympus opts systems, India). Glut-1 immunoexpression was analyzed from 0 - 3 grades, with '0' to represent negative staining (less than 10% positive tumor cells), 1 (10 - 25% positive tumor cells), 2 (25 - 50% positive tumor cells) and 3 (more than 50% positive tumor cells) to represent mild, moderate and intense staining respectively depending on the percentage of tumor cells that expressed the protein.

Statistical analysis

Glut-1 expression was assessed in both groups using Pearson's chi square test. All statistical steps were performed using Microsoft Excel and STATA software (Version 13). Pearson's chi square test was used to assess the correlation between grade of Glut-1 expression and evidence of metastasis. The correlation between Glut-1 expression and tumor stage was also assessed using Pearson's chi square test.

Results

Glut-1 expression was indicated by a more intense staining in the metastatic group (Figure 1) as compared to nonmetastatic group (Figure 2). Less intense staining was seen in central tumor cells as compared to peripheral tumor cells (Figure 3).

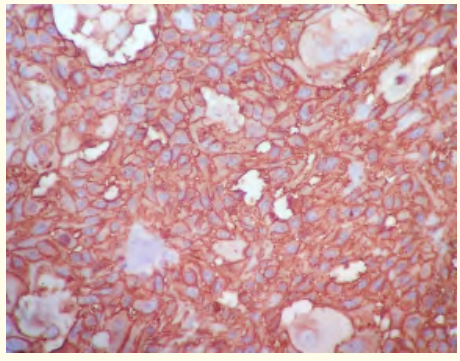

Figure 1: More intense staining of Glut-1 in oral squamous cell carcinoma from Group 1 (Immunohistochemical stain, Magnification:400X).

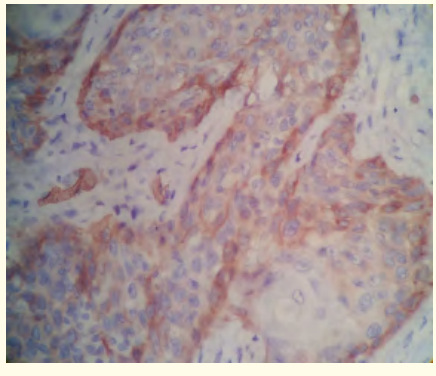

Figure 2: More intense staining of Glut-1 in oral squamous cell carcinoma from Group 2 (Immunohistochemical stain, magnification:400X).

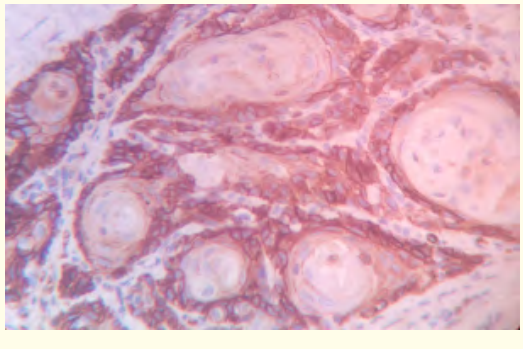

Figure 3: Less intense staining seen in central tumor cells as compared to peripheral cells in oral squamous cell carcinoma (Immunohistochemical stain, Magnification:400X).

Pearson's chi square test showed that there was no significant correlation between tumor grade and Glut-1 expression ($p > 0.001$) (Figure 4). Similarly, there was no significant correlation between pathological stage of the tumor and grade of Glut-1 immunoexpression ($p > 0.001$) (Figure 5). Pearson's chi square test showed that there was statistically significant correlation ($p = 0.001$) between grade of Glut-1 expression and metastasis as around 96.7% cases were showing grade 3 of Glut-1 expression in the metastatic cases and 60% cases were showing grade 3 of Glut-1 expression in non-metastatic group (Figure 6).

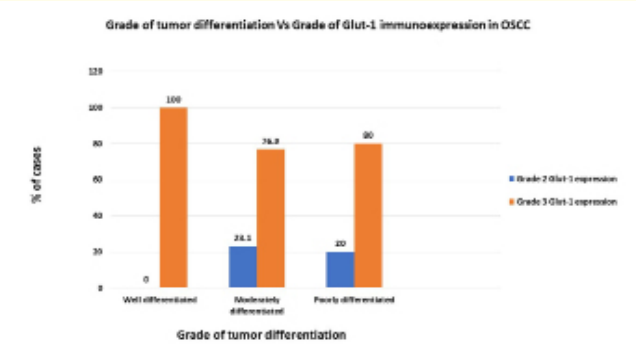

Figure 4: Graph showing grades of Glut-1 expression in various grades of oral squamous cell carcinoma.

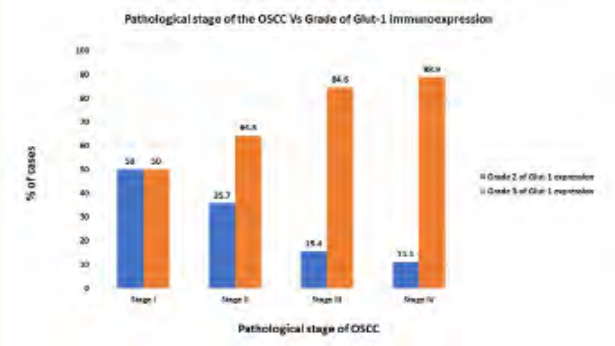

Figure 5: Graph showing increasing intensity of Glut-1 expression in oral squamous cell carcinoma with progression in tumor stage.

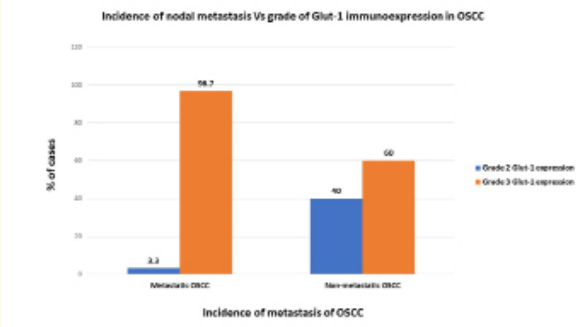

Figure 6: Graph showing increasing grade of Glut-1 expression with evidence of lymph node metastasis in oral squamous cell carcinoma.

Discussion

In several studies it has been shown that solid tumors contain poorly vascularized regions, low pH, severe hypoxia and nutrient starvation. There may be an increased requirement of oxygen, compared to surrounding tissues owing to an increased proliferation of tumor cells in solid tumors. Some studies have also evaluated the correlation between increased uptake of FDG, a glucose analogue, and the increased expression of Glut-1 in tumors [6-8]. The expression of Glut-1 has been found to be regulated by hypoxia in a HIF-1-dependent manner, which would thereby promote tumor progression. Fewer studies have been done on the importance of Glut-1 in the context of oral cancer. Few studies have been done on the role of glucose transporters and their effects on the tumor growth and further metastasis. Thus, the present study was aimed at determining the expression of Glut -1 in oral squamous cell carcinoma cases with and without regional lymph node metastasis.

Expression of Glut-1 increases under hypoxemic conditions which induces a shift in glucose metabolism towards glycolysis, which is brought about by HIF-1α expression in hypoxemic tumor microenvironment. Based on several studies, glucose transporter expression in different tumor systems has indicated an important role for Glut-1 during neoplastic transformation and tumor progression. Up-regulation of Glut-1 expression points towards an increased glucose consumption required for rapid proliferation of tumor cells. Thus, the present study was aimed at evaluating the correlation between Glut-1 expression and metastasis by studying its expression in the invasive tumor front.

The present study was a retrospective comparative study. In the present study, no significant correlation was seen between factors such as age, gender, site of tumor and grade of tumor with metastasis. Grade of Glut-1 immunoexpression was determined in each case and the attempt was made to correlate it with histological grade of the tumor, evidence of lymph node metastasis and TNM stage of the case. These findings were in contrast to few previous studies as there was no significant correlation between grade of Glut-1 expression and tumor stage and tumor grade [9-14].

The present study used a similar grading system to evaluate Glut-1 expression as that of Harshani., *et al*. [3]. Mainly peripheral staining was seen in tumor islands and absent in the central keratin pearls i.e. a prostromal pattern. These findings were similar to the study of Angadi., *et al* [15].

In the present study Glut-1 expression was not found to increase with respect to grade of tumor and tumor stage which was similar to the results found by Demeda., *et al*. and Carvalho., *et al* [16]. Several other studies also showed similar results [9-14]. However, these findings were in contrast to few previous studies [3,5,15].

This can be explained by the fact that different grading systems are used in different studies Histological grading of oral squamous cell carcinoma can be carried out by using various grading systems. There is no universally accepted grading system. In present study, WHO grading system was used for grading which considers only keratin formation by tumor cells and no other parameters are considered for grading.

Tumor stage is determined by tumor size, extent of nodal metastasis and evidence of distant metastasis. Thus, increase in the size of the tumor and increase in the number of metastatic nodes will increase the stage of the tumor. In our study, the correlation between grade of Glut-1 immunoexpression and metastatic potential of tumor was statistically significant. However, as mentioned above, nodal metastasis is not the only parameter which signifies the correct stage of the disease. Nevertheless, the metastatic potential of the tumor certainly influences long term survival of the patient. Thus, it can be said that the tumors with strong immunoexpression of Glut-1 show more propensity for nodal metastasis which ultimately results in worse prognosis.

Present study shows that Glut-1 immunoexpression can be certainly useful for prognostic prediction of oral squamous cell carcinoma cases in future. Future researches with larger and more defined sample size may definitely show more predictable results. Additionally, the status of Glut-1 in tumors arising in individuals with glut-1 deficiency syndrome remains to be illustrated. Glut-1 deficiency syndrome occurs due to impaired glucose transport in the brain. This results in various central nervous system manifestations such as infantile seizures, developmental delay, acquired microcephaly, muscular abnormalities and complex movement disorder consisting ataxia and dystonia [17]. Hypothetically, tumors occurring in persons suffering from this disorder should have better prognosis as there will be deficient glucose transport across the cell membrane as a result of deficiency of Glut-1. However, this has to be validated with in vitro experiments also. Such experiments in future may also open new horizons for utilization of glut-1 as a therapeutic target. Thus Glut-1 has strong potential as prognostic and therapeutic immunomarker for OSCC cases in future.

Conclusion

The present study was a retrospective comparative study. The results of the present study indicate that immunoexpression of Glut-1 is stronger in metastatic cases of OSCC than cases without metastasis. Thus Glut-1 can be a useful biomarker for determining the metastatic potential and prognosis of OSCC cases.

Conflict of Interest

Nil.

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